932,662

PATENT SPECIFICATION

DRAWINGS ATTACHED

932,662

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COMPLETE SPECIFICATION

Therapeutic Compositions comprising Butyrospermol

We, LABORATOIRES LAROCHE NAVARRON, a French Body Corporate of 63, Rue Chaptal, Levallois (Seine), France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention has for its object a new therapeutic composition endowed with hormonal properties, and moreover cicatrizing and bactericidal properties.

According to this invention it has been discovered that butyrospermol, until now without practical application, possesses these various properties to a marked extent, making it an interesting therapeutic agent.

Butyrospermol, or 3β-hydroxy-(13α, 14β, 17β H)-lanosta-7,24 diene is a tetracyclic tri-terpene alcohol of formula $C_{30}H_{50}O$ (MW = 426.7) having the structural formula illustrated in the drawing accompanying the Provisional Specification.

It is extracted from the kernals of the fruit of *butyrospermum Parkii* a large tree common in West Africa, especially in the Sudan area, and known under the name of *karite* (shea-butter). It may equally well be extracted from the oil mill cakes of karite, in which it remains for the major part, or separated from the latex of the breadfruit tree, *Artocarpus integrifolia* (Jack fruit).

The raw material (shea-butter) after grinding is extracted with carbon tetrachloride. After evaporating the solvent, the whole of the collected fatty materials is taken up by sodium hydroxide dissolved in pure methyl alcohol. The fatty acids, especially the palmitic acid, are thus converted into a soap insoluble in chloroform. After the methyl alcohol has been driven off, the resultant soap is put in a chromatographic column and extracted with chloroform until the solvent no longer gives a coloration by addition of Noller's reagent (antimony trichloride in thionyl chloride solution). By evaporating the resultant chloroform solution, a mixture of triterpene alcohols is obtained: butyrospermol, β-amyrin and "Parkeol", of which mixture butyrospermol is the main constituent. Parkeol is a triterpene alcohol of formula $C_{30}H_{50}O$, the systematic name of which is 3β-hydroxy lanosta-9 (II), 24 diene. This mixture may thus be used as it stands for pharmaceutical purposes. However, if pure butyrospermol is desired, this mixture may be acetylated by acetic anhydride in the presence of pyridine. The acetates are then collected and may be separated either by virtue of their differences in solubility and hence by fractional crystallization, or by a fresh chromatography run on alumina. Upon separating the acetates, pure butyrospermol may be obtained by hydrolysis of its acetate.

Butyrospermol has the following characteristics:

m.p. = 108—113°C.

$[\alpha]_D$ = −12.5° in chloroform solution.

It gives a yellowish-brown coloration with tetranitro-methane, a reddish-brown coloration accompanied by a characteristic green fluorescence with Liebermann-Bouchard reagent, and a pink coloration changing to purple with Noller reagent.

Butyrospermol gives *inter alia* the following derivatives:

—Acetate: m.p. = 146—148°C.
$[\alpha]_D$ = +11.5°±2.5° in chloroform solution

—Benzoate: m.p. = 130—133°C.
$[\alpha]_D$ = +33.5° in chloroform solution

— Cinnamate and palmitate, the latter having the advantage of being readily soluble in fatty materials:

— Butyrospermone or 3-oxo-(13α, 14β, 17β H)-lanosta-7,24-diene
m.p. = 77—84°C.
$[\alpha]_D$ = −40°±4° in chloroform solution.

The pharmacological properties of butyrospermol will now be discussed. It has a threefold activity: it has harmonal, cicatrizing and bactericidal properties.

I — HORMONAL ACTIVITY:

This is mainly a cortico-suprarenal activity. It is related to the action of desoxycorticosterone (DOC-like-action) and to the action of cortisone (cortisone-like-action).

1. — DOC-like action:

This action, in contrast with the action of desoxy-corticosterone is also effective on oral administration; it is evidenced by means of the following test. The survival without aggression is determined for male rats weighing 40 g and having undergone suprarenalectomy. The animals are divided into two groups of 30, maintained at 30°C, and receiving at will balanced feed and, as drink, an aqueous solution of NaCl at 9‰. However, this beverage is discontinued after 21 days of test. The first group is used to control, and the second group is administered butyrospermol, by gastric tube, twice daily. Table I shows the percentage of survivors in each group, with respect to time (in days) following suprarenalectomy.

TABLE I

Days of test	Controls %	Butyrospermol, 250 γ daily by gastric tube — %
7th		
8th		
9th	100	
10th	87	100
11th	87	86
12th	87	71
13th	87	71
14th	50	71
15th	12	71
16th	12	71
17th	12	71
18th	12	71
19th	12	71
20th	12	71
21st	12	71
	Suppression of salt water	
22nd	12	71
23rd	12	71
24th	12	57
25th	12	43
26th	0	28
27th		28
28th		28
29th		28
30th		28
31st		0

2. — Cortisone-like action:

This action is evidenced by the survival test of male rats weighing 35 g, subjected to cold (+3°C.) 48 hours after having undergone suprarenalectomy.

The rats are divided into three groups of 20. Two of the groups are administered, at regular 90 minute intervals, sub-cutaneous injections of 1 ml of water containing 10% alcohol and 5γ and 500γ, respectively, of butyrospermol. The third group is used as control and is only administered the vehicle, at the same time intervals.

The Table II given farther shows, for each case, the percentage of surviving animals with respect to time following the beginning of the experiment.

TABLE II

	Controls	Butyrospermol	
		5 γ	500 γ
4 h	100		
15	80		
30	60		
45	60		100
5 h	60		83
15	60		83
30	60		83
45	60		83
6 h	60	100	83
15	60	80	83
30	60	80	83
45	40	80	83
7 h	40	80	50
15	40	60	50
30	40	40	33
45	40	0	33
8 h	40		16
15	20		16
30	0		0
45			
Mean time of survival	6 h 27	7 h 16	7 h 10
% of increase over controls		+12.27%	+11.10%

II — CICATRIZING PROPERTIES:

1. Cicatrization test on the cornea of rabbit.

Similar trauma of the cornea, by means of a red-hot iron, are carried out on rabbits of same origin, of same weight, and having previously been submitted to local anaesthesia.

A first group of rabbits is used as control.

A second group of rabbits is given intramuscular injections of butyrospermol, which is administered twice weekly at a dosage of 30 mg. After 10 days, cicatrization is complete only in the rabbits which have been treated, and the cornea recovers its transparency.

2. Cicatrization test on experimental wounds in mice.

The rate of cicatrization and the death rate of male mice (25 g) submitted to a local application of 0.010 g of butyrospermol three times weekly is determined by comparison with untreated controls.

The resulting data are shown in Table III; the abbreviations have the following meanings:
IC : Index of cicatrization, percentage of the surface covered as compared to the original surface of the wound:
M : percentage of mortality.

TABLE III

Day following the day of experimental injury	10th		20th	
	I.C.	M.	I.C.	M.
Controls (20 subjects)	49	20	72	40
Treated (20 subjects)	78	15	89	15

III — Bacteriostatic action:

Butyrospermol has bactericidal properties with respect to acid-resistant bacilli, especially with respect to Koch bacillus and to Hansen bacillus *in vitro*.

Following a first phase of normal, sometimes accelerated, developement, a solution of butyrospermol causes a marked inhibition of cultures of Koch bacilli or of Hansen bacilli from a 0.015 g/ml concentration.

The hormonal properties of butyrospermol may be advantageously used in the following cases: secondary suprarenal insufficiency, Addison's disease or ovarian insufficiency. Butyrospermol is also effective as an adjuvant of the follicular steroids.

Butyrospermol may also be used for its cicatrizing properties with respect to wounds either on local or on general administration. Finally, butyrospermol is active against the bacilli of leprosy and tuberculosis of the skin, as against all the Grampositive cocci (staphylococci and streptococci).

For these various uses, butyrospermol may be incorporated into pharmaceutical compositions in association with a pharmaceutically administrable vehicle, which, when liquid, is sterile.

This vehicle depends upon the method of administration, this generally being systemic, although topical application is also possible.

When administered systemically, butyrospermol may be given in a dose of 0.100 to 0.500 g daily, either orally in association with a solid vehicle, or parenterally in association with a sterile liquid vehicle.

Thus, for oral administration, tablets each containing for example, 0.050 g of the active compound in association with the usual excipients will be advantageously used.

Compositions for parenteral administration may be made up in two separate parts, to be mixed immediately before use, namely:
— a sterilized bottle containing for example 0.050 g of sterile butyrospermol,
— an ampoule of 1 ml of sterile solvent.

For topical application, the butyrospermol may be made into balm and ointment formulations by incorporating it into the usual pasty vehicles.

WHAT WE CLAIM IS:—

1. A therapeutic composition comprising as active compound in association with a pharmaceutically administrable solid, semi-solid or sterile liquid vehicle, butyrospermol 3β-hydroxy - (13α, 14β, 17β H) - lanosta - 7,24-diene having the formula illustrated in the drawing accompanying the Provisional Specification.

2. A composition as claimed in claim 1, in tablet form, the vehicle being a pharmaceutically acceptable inert solid.

3. A composition as claimed in claim 1, characterized for injection formulated as two parts to be mixed extemporaneously and comprising:
— a sterile bottle containing sterile butyrospermol.
— an ampoule of sterile solvent.

4. A composition as claimed in claim 1, formulated as a balm or ointment, the vehicle

being a pasty excipient for topical applications.

5. A therapeutic composition according to claim 1, substantially as described.

MARKS & CLERK,
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932662 PROVISIONAL SPECIFICATION
1 SHEET *This drawing is a reproduction of the Original on a reduced scale*